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INHERITANCE OF STRIPE RUST RESISTANCE IN SOME EGYPTIAN WHEAT CULTIVAR

Sherif, S.E.; A. A Abu Aly and M. A.Hasan
Plant Disease Rest. Lab. Giza Agric. R. Sen. Egypt.

ABSTRACT

Stripe rust (yellow rust), caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most devastating foliar diseases of wheat (*Triticum aestivum*) worldwide. Growing resistant cultivars is the best approach for control of the disease. but only a few genes confer effective all-stage resistance against the current populations of the pathogen worldwide. It is urgent to identify new genes for diversifying sources of resistance genes and for pyramiding genes for different types of resistance in order to achieve high levels of durable resistance for sustainable control of stripe rust. The objective of this study was to identify Inheritance of stripe rust resistance in some Egyptian wheat cultivars.

Five crosses between Sakha 61 and each of Sakha 69, Giza 163, Gemmiza 5 Sids 7and 7 Sids 8 were performed. Seedlings of the parents , F1 and F2 were tested with *P. striiformis* f. sp. *tritici* races 172E155 under controlled greenhouse conditions. Crosses tested at seedling stage exhibited susceptible reaction against stripe rust. Under field condition plants segregation indicated that F_1 plants of the five crosses were resistant and exhibited low stripe rust severity ranged between 0 and 20 R. The result of F_2 plants reaction exhibited wide range of stripe rust severity ranged between 0 and 60 S but the direction was in the side resistance this confirmed the results of F_1. This study indicated that c.v. Sakha 61 also contains the stripe rust resistance gene at adult stage such as the tester proved to have.

The sergeaint analysis in amplified DNA polymorphism of tested wheat individuals using the primer (GACCGCTTGT) clearly showed that resistance gene was present in parent sakha61,f1and F_2 of resistance and was absent in f2 susceptible.

Key words : Wheat , Stripe rust , Resistant genes

INTRODUCTION

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most important diseases of wheat in the world. In Egypt, the last dramatic epidemic was, stripe rust attacked most of the commercial wheat cvs.in 1995 causing severe infection particularly in North and South Delta districts . stripe rust caused high loss in the production of most Egyptian wheat cultivars in the Delta area during 1996/1997 growing season El-Daoudi, 1998. recently the development of molecular marker for specific gene allows the detection of these genes independently of the genotype .Molecular markers can be used as marker assist selection for an effective combination of genes in pyramiding strategy to create more durable resistance Rolefs et al.,1992. Also, the objective of this study was to identify the stripe rust resistance genes in crosses of certain wheat population.

MATERIALS AND METHDOS

five wheat cultivars i.e Sakha 69, Giza 163, Gemmiza 5 Sids 7 and Sids 8 exhibited high susceptibility to stripe rust, while the cultivar Sakha 61 of resistance to stripe rust at adult stage (Aly, 1999). These parents were sown at Sakha Agric. Res. St. during 2008/2009 growing season in five rows each. All possible crosses among the five cultivars and Sakha 61 were conducted to produce the hybrid seed of the five crosses. The resulted F_1 plants are represented as follow: Sakha 61 x Sakha 69, Sakha 61 x Giza 163, Sakha 61 x Gemmiza 5, Sakha 61 x Sids 7, Sakha 61 x Sids8 during 2008/2009 growing season, part of the five F_1 hybrid seed was sown to produce the F_2 seed. The rest were left for the final experiment in the next season 2009/2010. An experiment was conducted in a randomized complete block design with three replicates each contained two rows for each parent and F_1 as well as 10 cm for each F_2. This performance was carried out to creat uniform environmental conditions. The rows were 3 m long 30 cm apart and seeds were sown 10 cm apart within rows. Therefore, each row contained 40 plants. Mixture of highly susceptible wheat cultivars were sown around the experiments as a spreader to disseminate the stripe rust urediospores of the pathogen (*Puccinia striiformis* f. sp. *tritici*). All regular cultural practices were precisely applied during the growing season, as recommended.

Ten pots for each of the parents and F_1 as well as 30 pots for each of F_2 plants were sown. Each pot contained 10 seed. Seedling (8 days-old) of the parents, F_1 and F_2 were uniformly inoculated with the urediniospores of physiologic race 172E155 which was used for inoculating all of the tested cross at seedling stage in the greenhouse using the technique described by Johnson *et al*. (1972). Infection type data against the pathogen were recorded after approximately 17 days of inoculation according to the scale described by McNeal *et al*. (1971).

- The infection types i.e. 0, 1 and 2 were considered resistant.
- 3, 4 and 5 types, moderate resistant or (intermediate sporulation).
- 6 and 7 moderate susceptible .
- 8, 9 high susceptible.

In the adults tested under field conditions, inoculation was restricted in the spreader plants which were moistened and dusted with spore mixture using the most prevalent stripe rust races. The inoculum was mixed at the rate of 1:20 (urediniospores to talcum powder) (w:w). All five crosses were inoculated at booting stage according to the method adopted by Tervet and Cassel (1951). Data of stripe rust severity % were recorded on adult plants according to Peterson *et al*. (1948). To study inheritance of resistance, the F_2 plants were grouped into two categories depending on the percentage of the disease severity and infection type under field conditions. The disease severity (%) i.e. 0, R and MR were considered as the resistance phenotypes, while MS and S were considered as the susceptible ones. Statistical and genetic analysis frequency distribution values were estimated for each of parents, F_1 and F_2 populations for infection type in all of the tested crosses in respect. To clarify, mode of inheritance of the expected ratio of the phenotype

classes of the stripe rust, infection types were determined using X^2 analysis according to the method of Steel and Torrie (1960). Molecular markers assigned for detection of stripe rust resistance genes in wheat were applied.

RAPD analysis

Sakha 61 resistant to stripe rust at adult stage was crossed to Giza163 susceptible and gave resistant in F_2 segregating plant . F_2 plants were evaluated at adult stage for stripe rust under field condition and fresh samples wer collected for RAPD analysis.

The specific primer was chosen according to the findings of Motawi *et al*. (2003) who tested 21 RAPD primers assigned for wheat stripe rust and found that only two (GAAACGGGTG) and (GACCGCTTGT) gave additional band to the resistance of Sakha 61. Only one of them was chosen herein viz (GACCGCTTGT)

Statistical analysis and goodness of fit to a 3:1 ratio was calculatred for RAPD marker using Chi-square (X^2) test.

RESULTS

The infection type, frequency distribution and the disease severity classes of the parents, F_1 and F_2 populations of each of the five crosses were performed. Inoculation at seedling was accomplished by using race 172E158 and a mixture of the most prevalent races in the area at adult stage

Data in Table (1) reveal the, all of parents ,F_1 and F_2 plants tested at seedling exhibited susceptible reaction against the physiologic race 172E158 (infection type8-9). While the five crosses between cultivar Sakha 61 showed no segregation at seedling stage. This result indicated that, these cultivars do not have the stripe rust resistance gene at seedling stage. The results of crosses between the five wheat cultivars and the stripe rust resistant cultivar Sakha 61 at adult stage are shown in table (2). Five parents exhibited high susceptibility, where stripe rust severity (%) ranged between 40s-60s. Meanwhile, Sakha 61 was highly resistant. As for F_1 plants of the five tested crosses exhibited high resistance, where their stripe rust severity (%) ranged between 0 and 10R. These results revealed that resistance was dominant over susceptibility in these crosses in F_1 at adult stage.

The obtained results derived from F_2 of the five tested crosses having resistance gene exhibited a wide range of reaction to stripe rust severity ranged between 0-60s. with expected ratio 3:1. This 3:1 ratio verified that single dominant gene pair control resistance and supported the fact that cultivar Sakha 61 carried the adult plant resistance gene and showed gene expression of resistance to stripe rust in all tested crosses at adult stage.

Table (1): Segregation for stripe rust Infection type in F2 of the five crosses against stripe rust race 172E155 at seedling stage.

Crosses and parents			Phenotypes		Expected ratio	X^2
			Res	Sus		
Sakha 61 x Sakha 69	P_1	50		50	-	-
	P_2	50		50	-	-
	F_1	40		40	-	-
	F_2	140		140	-	-
Sakha 61 x Gemmeiza 5	P_1	50		50	-	-
	P_2	50		50	-	-
	F_1	35		35	-	-
	F_2	160		160	-	-
Sakha 61 x Giza 163	P_1	50		50	-	-
	P_2	50		50	-	-
	F_1	40		40	-	-
	F_2	145		145	-	-
Sakha 61 x Sids 7	P_1	50		50	-	-
	P_2	50		50	-	-
	F_1	40		40	-	-
	F_2	150		150	-	-
Sakha 61 x Sids 8	P_1	50		50	-	-
	P_2	50		50	-	-
	F_1	40		40	-	-
	F_2	140		140	-	-

Res = Resistant , Sus = Susceptible

Table (2): Segregation for stripe rust severity (%) in F2 of the five crosses against Stripe rust using a mixture races at adult stage

Crosses and parents			Phenotypes		Expected ratio	X^2
			Res	Sus		
Sakha 61 x Sakha 69	P_1	70	70			
	P_2	70		70		
	F_1	45	45			
	F_2	142	94	48	3:1	1.96
Sakha 61 x Gemmeiza 5	P_1	70	70			
	P_2	70		70		
	F_1	37	37			
	F_2	202	151	51	3:1	0.56
Sakha 61 x Giza 163	P_1	70	70			
	P_2	70		70		
	F_1	50	50			
	F_2	185	129	56	3:1	0.76
Sakha 61 x Sids 7	P_1	70	70			
	P_2	70		70		
	F_1	46	46			
	F_2	222	165	57	3:1	0.50
Sakha 61 x Sids8	P_1	70	70			
	P_2	70		70		
	F_1	38	38			
	F_2	240	173	67	3:1	0.095

Res = Resistant , Sus = Susceptible

The detection of resistance genes in wheat crosses using the molecular markers, data in Table (3) and illustrated in Fig. (1) revealed that the produced DNA bands of tested wheat individuals, clearly showed that the bands of Sakha 61 are present in lane (the consequence of bands) and the two individuals of F_2 plants are only linked with the primer and rendering as specific clear bands (700 pb) with the exception of (lane 1) and one susceptible individual of F_2 derived from Sakha 61 x Giza163 (lane 4).

Meanwhile, Giza163 did not link with the primer at (700 pb) and could not be detected.

The analysis of this polymorphism revealed that only 31 out of 46 individuals of F_2 have linked with the primer where the rest 15 individuals did not. This result revealed that the resistant susceptible individuals are 31 with expected ratio 3:1 which verified by X^2. This result confirmed the presence of resistance gene in the segregation of the resulted crosses and verified that a single dominant gene pair controls resistance.

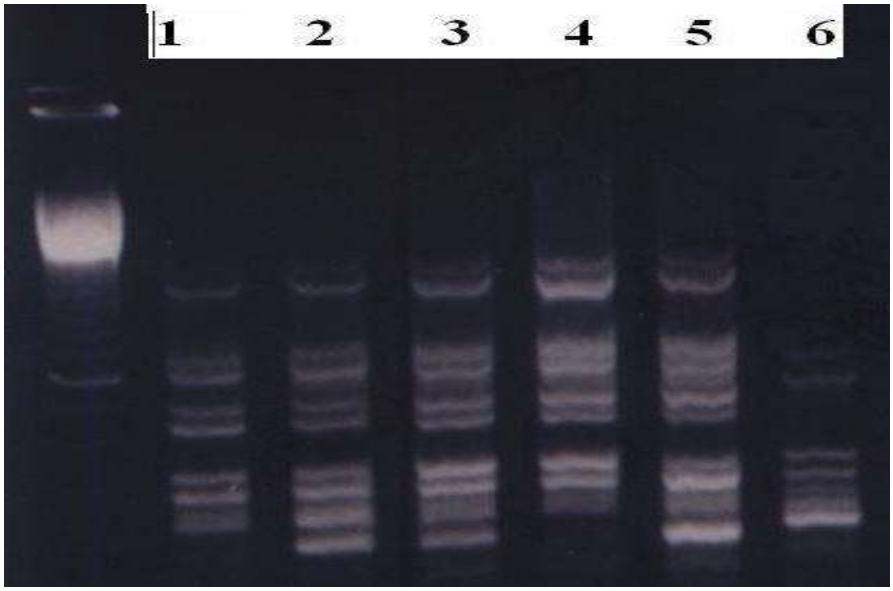

Fig. (1): RAPD- DNA polymorphism of parents,f1 and F_2 segregates having cv. Sakha 61 as resistant gene and linked with primer (GACCGCTTG) Giza 163 (Lane 1), Sakha 61 (Lanes 2), F_1 (3), susceptible f2 (lane4) and resistance f2 (lane 5 and 6) (M) Molecular weight of marker . The arrowhead indicated the bands at 700bp which differentiating between susceptible and resistant cross.

Table (3): RAPD marker linked with cv. Sakha 61 in resistant wheat crosses segregation of F_2

RAPD markers	Tested crosses	Phenotypes		Expected ratio	X^2
		R	S		
700 pb	Sakha 61 x Giza 63	31	15	3:1	0.43

DISCUSSION

Most of the Egyptian wheat cultivars exhibit considerable level of susceptibility, with few exception EI-Dauodi *et al*. (1998) studying five crosses to stripe rust infection at seedling stage under greenhouse condition showed

susceptibility to stripe rust (infection type 8-9). However, F_2 populations did not fit any ratio , which may be due to the phenomenon of partial suppression of resistance genes. At adult stage Sakha 61 and its crosses with tested cvs. (F_1) exhibited high resistance .F_2 segregations of crosses having Sakha 61 showed that resistance was dominant over susceptibility. The results indicated that crosses fitted the expected ratio 3:1. This ratio verified that single dominant gene pair control stripe rust resistance and supported the F_1 result at adult stage. Molecular marker can be used as marker assists selection for an effective combination of genes and in a pyramiding strategy to create more durable resistance Rolefs *et al*., 1992. Motawi *et al*. (2003) develop molecular marker from the Sakha 61 DNA sequence which was very specific for this cultivar resistance gene in breeding material of diverse genetic origin. The produced random amplified DNA polymorphism (PAPD) of tested wheat individuals using the primer (GACCGCTTGT) clearly showed that cv. Sakha 61 carried a gene which was successfully transferred present in F_2 of Sakha 61 x Giza 163 which showed resistance and absent in susceptible individuals. This result confirmed the presence of resistant gene in the segregations of the resulted crosses and verified that a single dominant pair gene controls stripe rust resistance at adult stage and supported the fact that cultivar Sakha 61 carried the adult plant resistance gene and showed gene expression of resistance to stripe rust in all tested crosses at adult stage. This work could be usefully applicable in the breeding wheat program against rust diseases in general and stripe rust in particular under Egyptian conditions.

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وراثة المقاومة للصدأ الأصفر فى بعض اصناف القمح المصرى

صلاح الدين شريف ,عبد العزيز عبد الناصر محمد ابو على و محمد عبد القادر حسن

قسم بحوث أمراض القمح ـ معهد بحوث أمراض النبات ـ الجيزة

يعتبر الصدأ الأصفر فى القمح المتسبب عن الفطر (*Puccinia striiformis tritici*) أكثر أمراض القمح خطورة فى مصر حيث تكرر ظهوره بحالة وبائية مسببا خسائر عالية فى المحصول مما أدى إلى إلغاء عدة أصناف تجارية لذا فقد دعت الحاجة إلى البحث عن مصادر المقاومة لهذا المرض تحتوى على عوامل وراثية ذات تأثير واسع وفعال.

كان الهدف من هذه الدراسة هو التعرف على وراثة المقاومة فى بعض اصناف القمح المصرى المقاومة للصدأ الاصفر لذلك تم التهجين بين الصنف سخا 61 المقاوم للصدأ الاصفر والأصناف القابلة لاصابة للحصول على الهجن الأتية (سخا 61 × سخا 69) ، (سخا 61 × جميزة 5) ، (سخا 61 × جيزة 163) ، (سخا 61 × سدس 7).و(سخا 61 × سدس 8).

اظهرت الدراسة فيما يتعلق بالتربية ضد العدوى بالصدأ الاصفر فى طور البادرة تحت ظروف الصوبة ان نباتات القمح فى الجيل الاول والثانى كانت قابلة للإصابة حيث تراوح الطراز المرضى المقدر مابين (8-9) وبالنسبة للتربية ضد الصدا الاصفر فى مرحلة النبات البالغ تحت ظروف الحقل أن نباتات الجيل الأول للهجن التى تحتوى على سخا 61 كانت كلها مقاومة. إذ ظهرت أقل نسبة إصابة والتى تراوحت بين الطراز المرضى (R 20-0) وقد أظهرت النتائج أيضا أن صفة المقاومة سائدة على صفة القابلية للإصابة فى الجيل الأول كما أظهرت نتائج الجيل الثانى مدى واسع من رد فعل النبات لمرض الصدأ الأصفر والتى تراوحت بين الطراز المرض (60S-0) ولكن كان اتجاه المقاومة للمرض هو السائد على القابلة للإصابة فى5 هجن ومؤكدا نتائج الجيل الأول. وهذه الدراسة توضح أن الصنف سخا 61 يحتوى على جين المقاومة للصدأ الأصفر فى طور النبات البالغ وكذلك الهجن التى تحتوى على سخا وتأكد حقيقة التعبير الجينى للصنف سخا61 فى طور البلوغ

كذلك أدى الكشف باستخدام طريقة RAPD-DNA عن وجود جين المقاومة فى أفراد هجين القمح المنعزل فى الجيل الثانى سخا 61 × جيزة 163 بإستخدام البادىء الوراثى المتخصص (GACCGCTTGT) الى تأكد وجود جين المقاومة فى الأفراد المقاومة المنعزلة ولم يكن موجودا فى الأفراد القابلة للإصابة وقد أكدت هذه النتيجة نسبة 1:3 وهذا يؤكد حقيقة التعبير الجينى للصنف سخا 61 فى طور البلوغ وكذلك الهجن التى تحتوى على سخا 61.

ويفيد هذا البحث تطبيقيا فى عمليات التربية للمقاومة للأمراض بصفة عامة ولأصداء القمح بصفة خاصة.

قام بتحكيم البحث

أ.د / محمد الششتاوى عبد ربه — كلية الزراعة – جامعة المنصورة

أ.د / مسرات الغمرى — مركز البحوث الزراعيه